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14. ABSTRACT

Current treatments for advanced prostate cancer are not very effective. To develop better treatment options, new approaches to investigate the signaling pathways important for advanced prostate cancer are warranted. Tyrosine kinase signaling contributes to numerous pathologies including cancer. In prostate cancer, mutation rates of tyrosine kinases are not readily observed, however, tyrosine phosphorylation of advanced prostate cancer is quite abundant indicating activity of tyrosine kinases are apparent even in the absence of activating mutations. Work supported by this award investigated this and found robust tyrosine phosphorylation in advanced prostate cancer in both human and mouse tumors. Using commonly perturbed non-tyrosine kinase oncogenes and pathways found in prostate cancer, phosphopeptide enrichment and quantitative mass spectrometry analysis revealed oncogene-specific tyrosine kinase signatures. Analysis of these signatures revealed unique tyrosine kinase signaling networks which may be targeted in advanced prostate cancer.

15. SUBJECT TERMS
Prostate cancer, metastasis, tyrosine kinase, signaling, phosphoproteomics, clinical, human

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Table of Contents

INTRODUCTION

Prostate cancer is the most commonly diagnosed and second leading cause of cancer-related death in American men (1). Death is usually a result of the spread of prostate cells to other sites in the body (metastasis) (2). Despite this high rate of mortality, little is understood about prostate cancer metastasis and in particular how to effectively treat this disease. Tyrosine kinase activity has been shown to play a role in prostate cancer, but targeting these same molecules clinically has not been very successful (3). The purpose of this grant was to provide important insights into which tyrosine kinases are crucial for advanced prostate cancer and, more importantly, help to elucidate better treatment strategies for this disease. The utilization of phosphopeptide enrichment and mass spectrometry will enable identification of these new targets in prostate cancer which may be investigated further for clinical benefit.

BODY

The specific aims of the award are listed below with updates on the training and research accomplishments.

Aim 1: Identify the specific tyrosine kinases activated during initiation and progression of genetically altered prostate cancer cells in the mouse.

We have made significant progress within this aim including a first author publication in *PNAS* (4). Parts a and b are completed and we are in the final stages of Part c.

a. Using our established vector system (Figure 1A), we generated a range of mouse prostate cancer phenotypes that display a distinct tyrosine phosphorylation profile (4, Figure 1B). These oncogene combinations are pertinent to prostate cancer including Akt, Akt+Erg, Akt+AR, and Akt+KrasG12V. We did find that Src/AR tumors were very difficult to generate enough material for phosphotyrosine evaluation so we were not able to pursue that particular oncogene combination.

b. Following tumor formation we evaluated each tumor phenotype using phosphotyrosine enrichment/mass spectrometry (4, Figure 2A). We were able to include all of the oncogene combinations listed above and normal mouse prostate as a control validated some of these kinases via western blot (Figure 2B).

c. We are currently evaluating this part of the aim. We have generated microRNAs that target 3 separate tyrosine kinases identified in Aim 1b. These include Src, Jak2, and Abl tyrosine kinases. These kinases were verified in the mouse tumors using western blot and immunohistochemistry (4). Using an inducible knockdown approach (Figure 3A), knockdown of these kinases has been validated after addition of doxycycline (Figure 3B). Further, we are simultaneously treating these tumors with the Src and Abl tyrosine kinase inhibitor Dasatinib to verify the functional importance of these two kinases in our mouse model system.

Aim 2: Investigate the tissue-specific tyrosine kinase activity in metastatic prostate cancer.

Parts a and b of this Aim are completed and Part c is nearly finished.

a. After introduction of luciferase into cells expressing an active SRC (Figure 4A), we introduced these cells subcutaneously into SCID mice and monitored growth and metastatic spread via bioluminescence imaging (Figure 4B). Over time, these mice developed lung and lymph node metastasis from subcutaneous prostate tumors expressing an active Src (Figure 4C). All tumors (subcutaneous and lung metastases) were excised and frozen for subsequent phosphopeptide enrichment/mass spectrometry analysis in Aim 2c.

b. Numerous 22Rv1 tumors were collected after intracardiac injection including metastatic tumors from the liver, lymph node, and lung cavity. These tumors were excised and frozen for subsequent phosphopeptide enrichment/mass spectrometry analysis. These tumors are being used in Aim 3b.

c. The tumors used in Aim 2a were analyzed separately from Aim 2b. In Aim 2a, the lung tumors derived from an activated SRC were compared to the primary tumor (Figure 5A). Distinct phosphopeptides were observed in the metastatic tumors when compared to the primary subcutaneous tumor and these targets are now being evaluated for relevance to human disease (Figure 5B). We are still evaluating these signatures in metastatic disease.

Aim 3: Identify the activated tyrosine kinases in advanced prostate cancer in man.

All parts of this aim is currently in progress. Part a has begun and we are still collecting samples for analysis. Part b will be completed once we have collected enough samples from Part a. We have not started Part c but plan to in the next 3-4 months.

a. We have made some significant progress in Part a. We initiated a collaboration with the lab of Ken Pienta (University of Michigan) to obtain metastatic prostate cancer tissue through their Warm Autopsy program (5). Collection of metastatic prostate cancer tissue is very difficult to obtain and so far we have acquired 4 samples, 2 of which were usable for phosphopeptide enrichment/mass spectrometry analysis based on histology (Figure 6).

b. We have run 2 metastatic prostate cancer samples, 3 primary prostates (controls), and the 22Rv1 metastatic tumors described in Aim 2b. This initial run identified 3 unique clusters of phosphopeptides, including a separate cluster for the metastatic samples (Figure 7). We are currently evaluating these kinases for functional significance and relevance.

c. This Part has not begun but we anticipate that we will be starting soon once we collect enough metastatic samples for analysis.

KEY RESEARCH ACCOMPLISHMENTS
- Identified robust tyrosine phosphorylation in advanced human prostate cancer.
- Generated a panel of mouse prostate cancer phenotypes that display different patterns of phosphotyrosine expression depending on the oncogene used to generate the tumor.
- Phosphotyrosine peptide enrichment and quantitative mass spectrometry identified oncogene-specific tyrosine kinase signatures, including activation of EGFR, ephrin type-A receptor 2 (EPHA2), and JAK2.
- Kinase:substrate relationship analysis of the phosphopeptides also revealed ABL1 and SRC tyrosine kinase activation.

REPORTABLE OUTCOMES
- Published a first-author manuscript in *PNAS* (4). (see appendices)
- Presented research findings at the Microbiology, Immunology, and Molecular Genetics Departmental Retreat (October 2011). (see appendices)
- Presented research findings at the AACR Advances in Prostate Cancer Research Conference in Orlando, FL (February 2012). (see appendices)
- Data deposition from the *PNAS* manuscript: MS2 spectra for all phosphopeptides reported in this paper have been deposited in the PRIDE database, http://www.ebi.ac.uk/pride/ (accession nos. 20879–20889).

CONCLUSION
The purpose of this award was to utilize different technologies to identify new therapeutic targets in advanced prostate cancer. Here, we have shown that we can identify oncogene-specific tyrosine kinase networks from mouse tumors and these networks implicate many tyrosine kinases which can be targeted therapeutically. While the mouse studies were straightforward, moving to human metastatic samples will be more challenging due to limited material and difference in genetic backgrounds. However, preliminary data suggests that phosphopeptides from metastatic tumors do indeed segregate from primary prostate tumors and cell lines. Deciphering this information will be crucial to better understand the mechanisms of metastatic prostate cancer.

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3. Araujo JC, *et al.* (2011) Dasatinib combined with docetaxel for castration-resistant prostate cancer: Results from a phase 1-2 study. *Cancer*.

4.   **Drake JM, *et al.* (2012) Oncogene-specific activation of tyrosine kinase networks during prostate cancer progression.** *Proc Natl Acad Sci U S A* **109(5):1643-1648.**
5.   **Rubin MA, *et al.* (2000) Rapid ("warm") autopsy study for procurement of metastatic prostate cancer.** *Clin Cancer Res* **6(3):1038-1045.**

APPENDICES (see attached)
- Supporting Data (Figures 1-7)
- Drake et al. *PNAS* Manuscript
- 2011 MIMG Departmental Retreat Abstract
- 2012 AACR Advances in Prostate Cancer Research Abstract
- 2012 AACR Advances in Prostate Cancer Research Program

Supporting Data

A

B
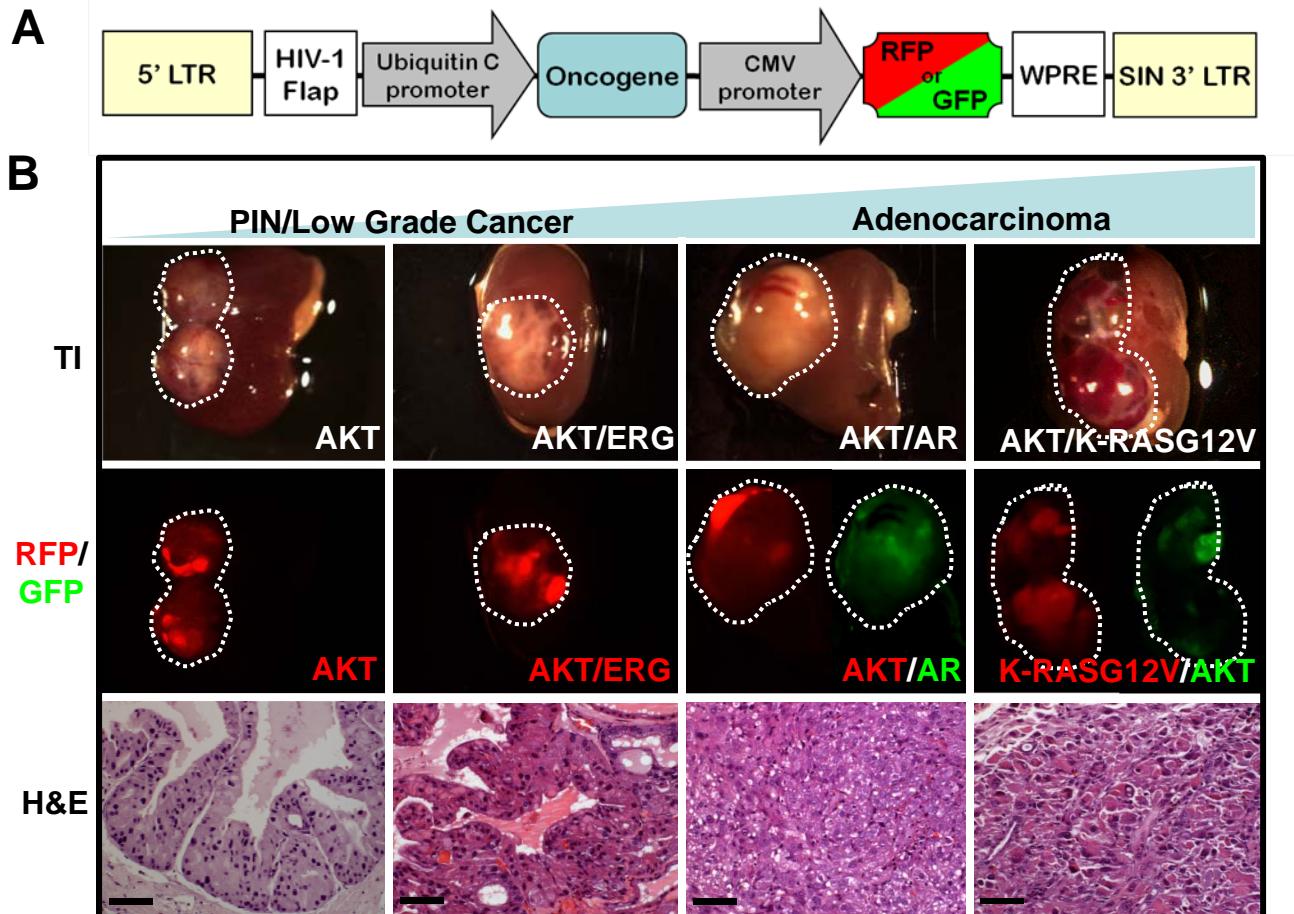
Figure 1. Phosphotyrosine expression is increased during prostate cancer progression. (A) Lentiviral vector diagram displaying the organization of oncogene and fluorescent marker expression used in these tumors. (B) Gross and histological morphology of each tumor type after 12 week engraftment in SCID mice using the prostate regeneration protocol. Fluorescence corresponds to expression of a particular oncogene. TI=transillumination, H&E=hematoxylin and eosin. Scale bars=50 μm.
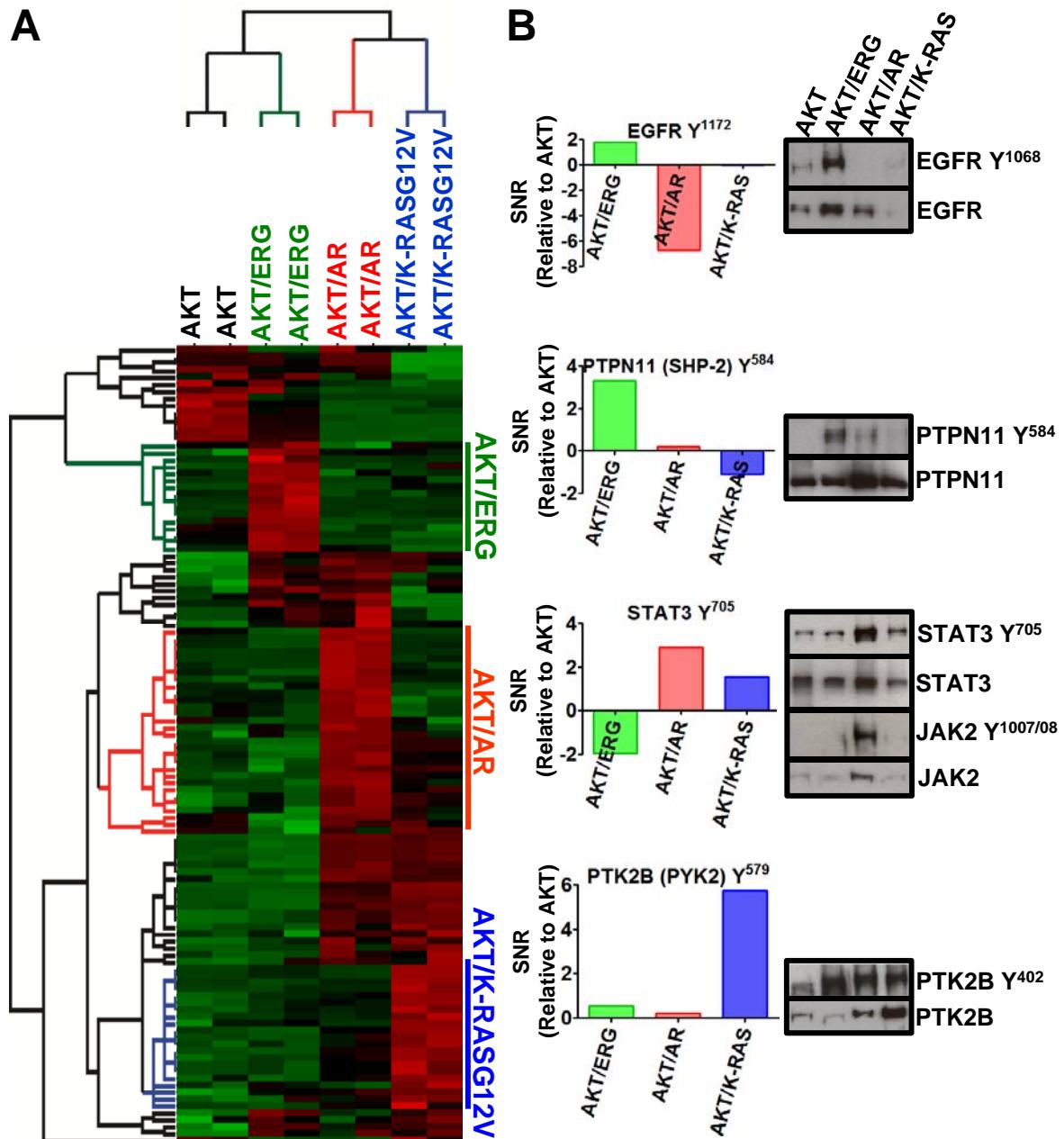
Figure 2. Unique phosphotyrosine signatures are observed in a mouse model of prostate cancer progression. (A) Heatmap representing unique clusters of tyrosine phosphorylation for each mouse tumor phenotype. Each row corresponds to a unique phosphopeptide. Red=hyperphosphorylation, green=hypophosphorylation for each phospho-peptide. (B) Specific tyrosine kinases are observed in an oncogene-specific fashion. Signal-to-noise ratio (SNR) (relative to AKT) was calculated for each phosphorylation event and plotted. Positive SNR confirms elevation of that particular phosphorylation event. Western blotting validates indicated oncogene-specific phosphorylation results.

A

B

miRNA: Scr.   ABL   JAK2   SRC   SRC/   JAK2/   SRC/   SRC/
                                    ABL   ABL   JAK2   ABL/
                                                          JAK2

Dox: - +   - +   - +   - +   - +   - +   - +   - +

ABL

JAK2

SRC

ERK2
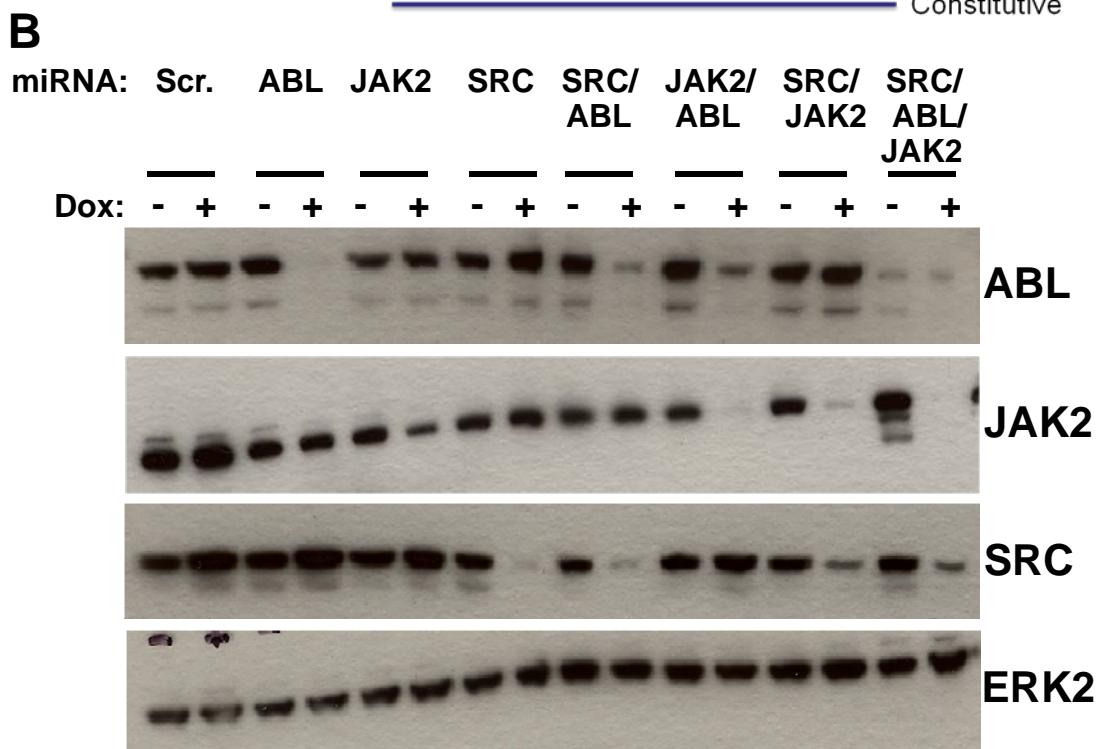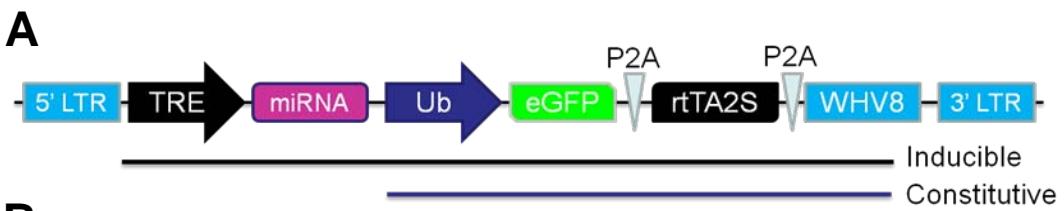
Figure 3. Micro-RNA silencing of mouse tyrosine kinases ABL, JAK2, and SRC using an inducible vector system. (A) Lentiviral vector diagram displaying the organization and inducible regulation of microRNA (miRNA) and constitutive fluorescent marker expression. (B) Western blot analysis confirms the knockdown of each of the targeted kinases, including chaining multiple different kinases on the same vector. Scr. = scrambled miRNA.
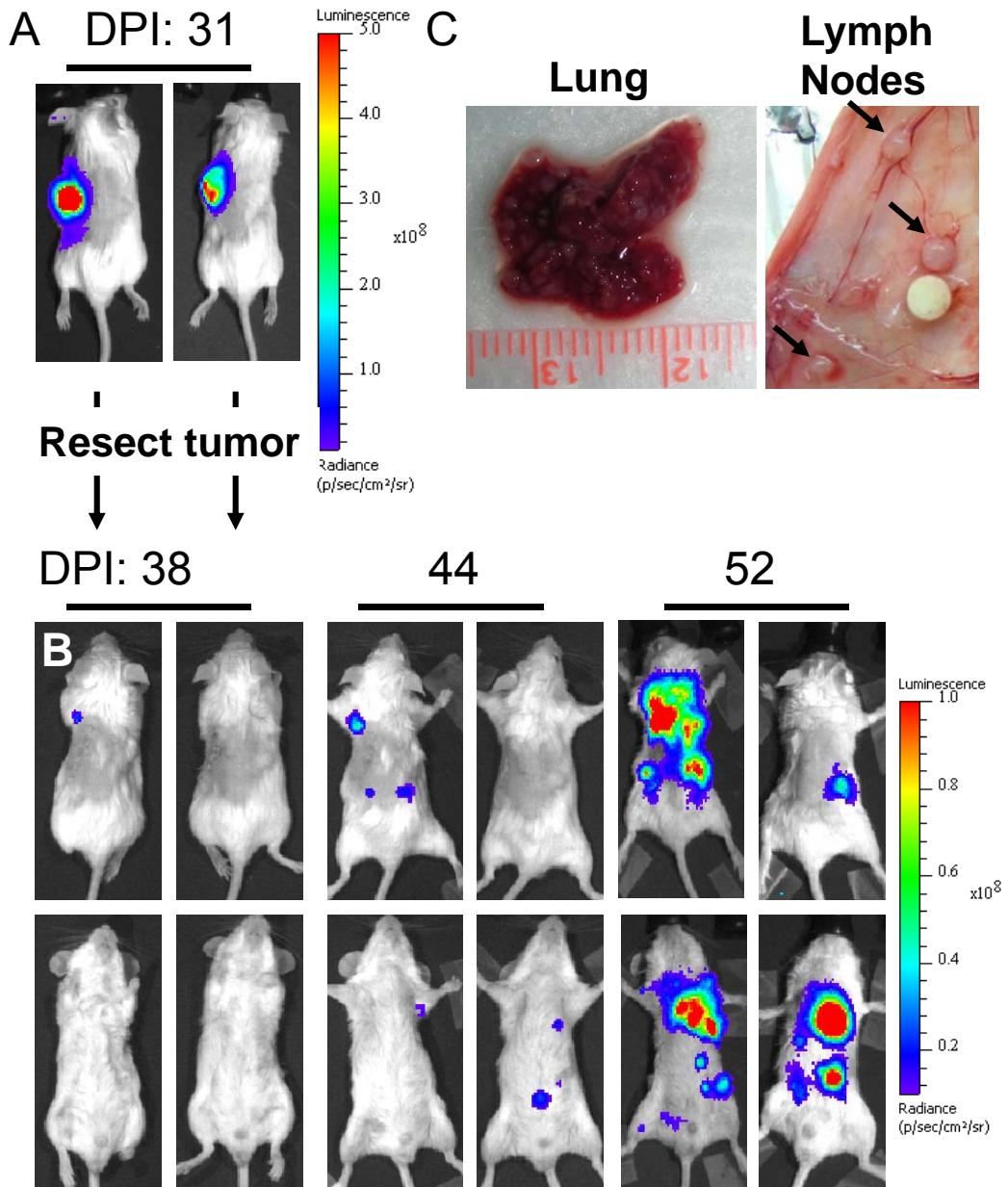
Figure 4. Subcutaneous implantation of cells expressing an active SRCc metastasizes to lung and lymph nodes. (A) After introduction of luciferase, SRCY529F (activated SRC) cells were implanted subcutaneously into the left flank of SCID mice and tumor growth was monitored using bioluminescence imaging. After 31 days post injection, the resulting tumors were then resected and metastatic disease was monitored. (B) Metastatic tumor growth was monitored over the course of 3 weeks revealing metastatic spread. (C) Lung and lymph node metastasis were evident after autopsy.
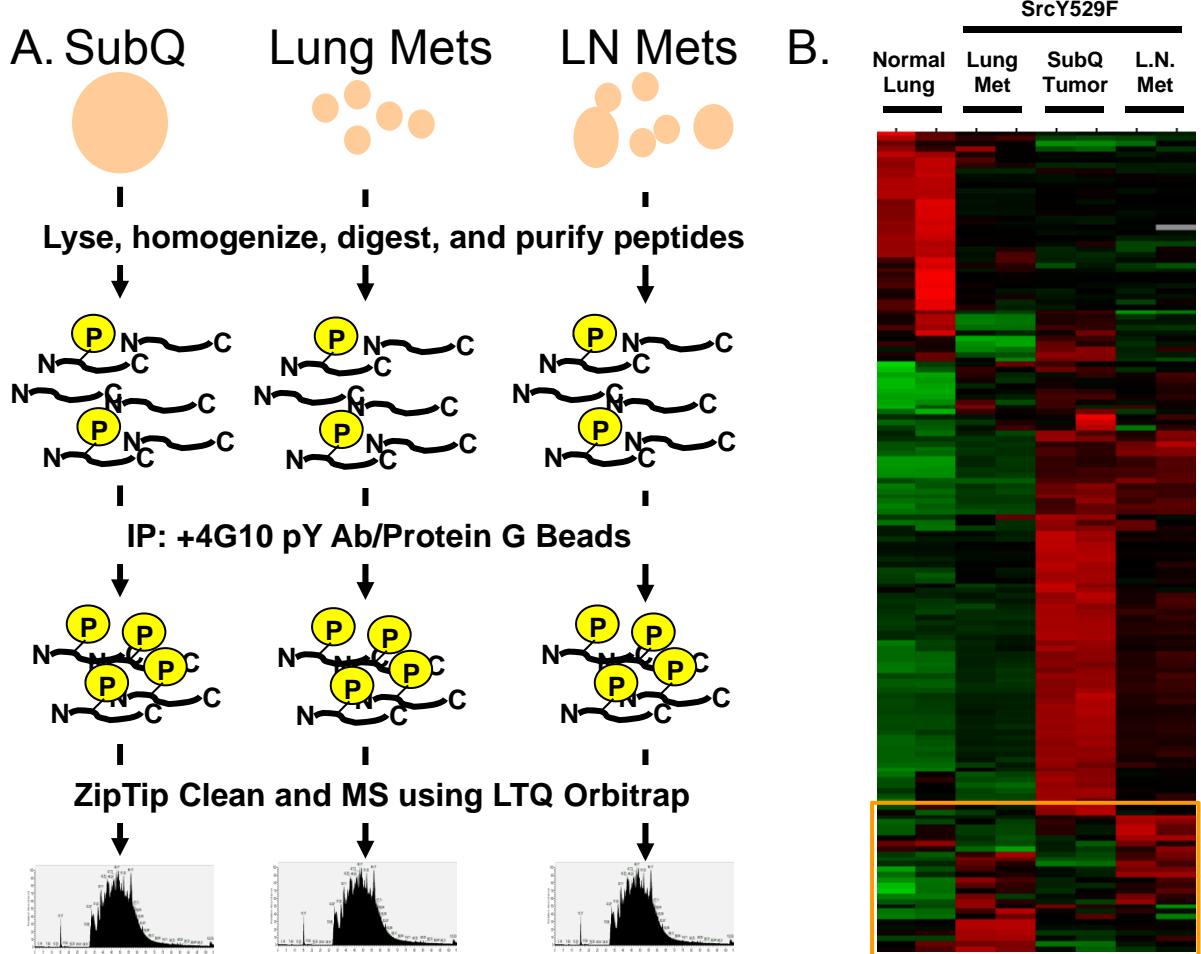
Figure 5. Tyrosine kinase profiling reveals a subset of proteins enriched in metastatic lesions. (A) Schematic of experimental design to enrich and purify tyrosine kinases and their substrates. Subcutaneous primary tumors and lung and lymph node metastases were compared for tyrosine kinase activity. (B) Heatmap analysis reveals a subset of identified tyrosine kinases and substrates to be enriched in the metastatic tumors (orange box).

Left Lung MET

Very Little Tumor <10%

H&E

Perihilar Lymph Node Tumor

Gleason 10 ~100%

H&E

Liver MET

Small Cell Carcinoma ~100%

H&E

Adrenal MET

Very Little Tumor <10%

H&E
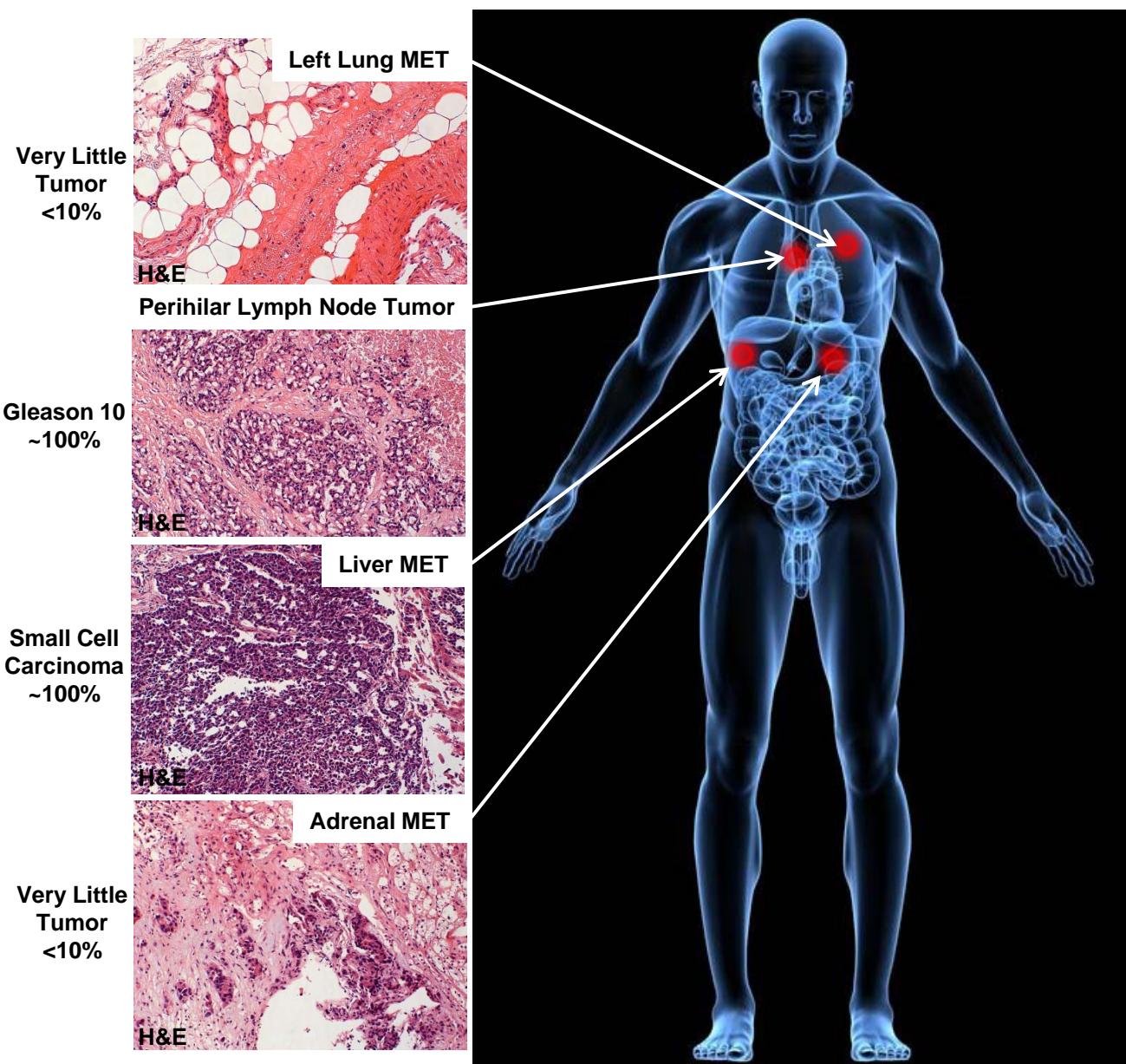
Figure 6. Initial set of metastatic samples obtained from rapid autopsy program for phosphoproteomic analyses. Schematic of metastatic locations and histological phenotype for each of the 4 metastati.c samples obtained from the rapid autopsy program and the lab of Ken Pienta at the University of Michigan. 2 samples (Liver Met and Perihilar LN Met) were suitable for further analyses.
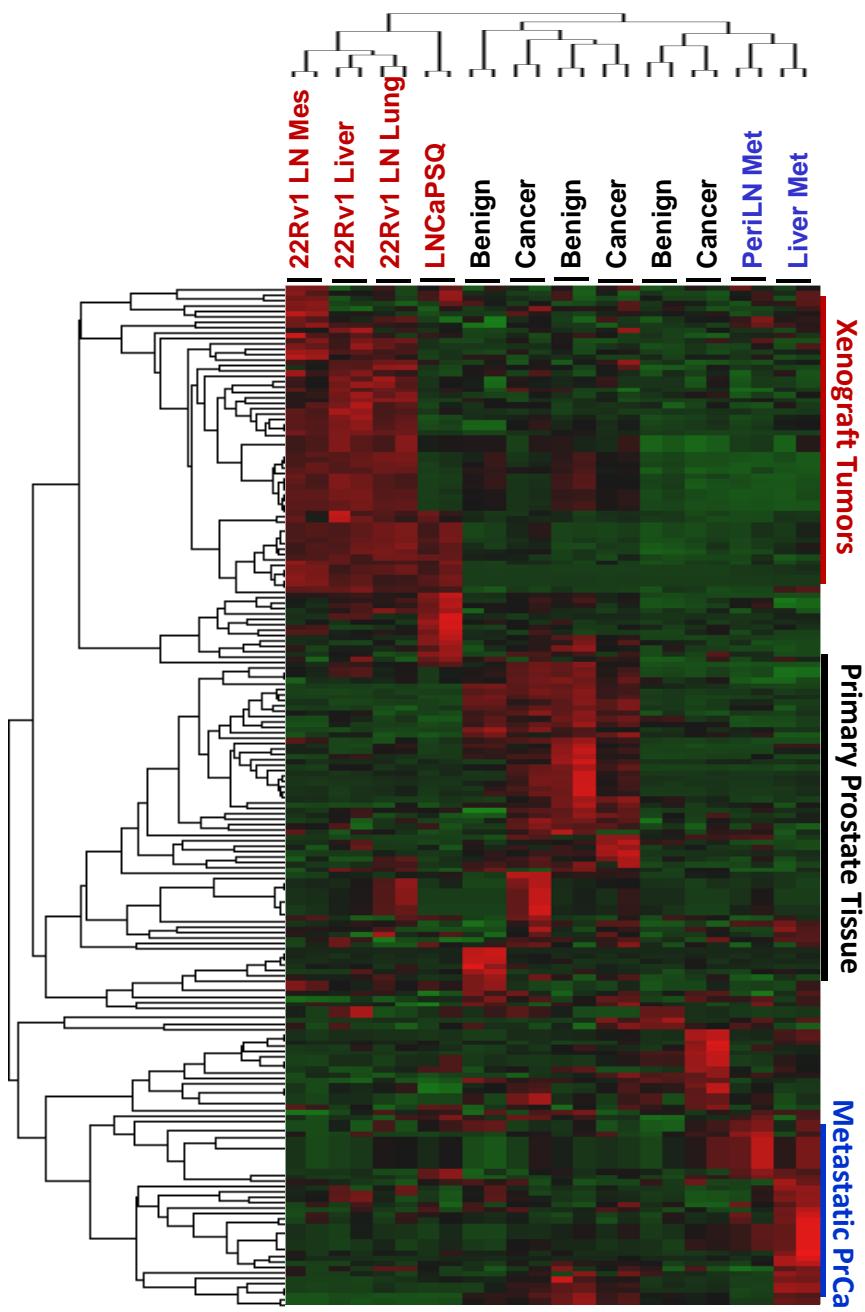
Figure 7. Phosphopeptide enrichment and mass spectrometry identified unique clusters within the metastatic samples. Three different sample clusters are observed in this data set including 1. Xenograft tumors from cell lines (red), 2. Primary prostate tissues (black), and 3. Metastatic tissues (blue). Each row corresponds to a unique phosphopeptide. Red=hyperphosphorylation, green=hypophosphorylation for each phospho-peptide.

Oncogene-specific activation of tyrosine kinase networks during prostate cancer progression

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Contributed by Owen N. Witte, December 20, 2011 (sent for review November 29, 2011)

Dominant mutations or DNA amplification of tyrosine kinases are rare among the oncogenic alterations implicated in prostate cancer. We demonstrate that castration-resistant prostate cancer (CRPC) in men exhibits increased tyrosine phosphorylation, raising the question of whether enhanced tyrosine kinase activity is observed in prostate cancer in the absence of specific tyrosine kinase mutation or DNA amplification. We generated a mouse model of prostate cancer progression using commonly perturbed non-tyrosine kinase oncogenes and pathways and detected a significant up-regulation of tyrosine phosphorylation at the carcinoma stage. Phosphotyrosine peptide enrichment and quantitative mass spectrometry identified oncogene-specific tyrosine kinase signatures, including activation of EGFR, ephrin type-A receptor 2 (EPHA2), and JAK2. Kinase:substrate relationship analysis of the phosphopeptides also revealed ABL1 and SRC tyrosine kinase activation. The observation of elevated tyrosine kinase signaling in advanced prostate cancer and identification of specific tyrosine kinase pathways from genetically defined tumor models point to unique therapeutic approaches using tyrosine kinase inhibitors for advanced prostate cancer.

AKT | androgen receptor | ERG | K-RAS | bioinformatics

The future of effective cancer treatment is based on the emerging concept of personalized therapy, which requires detailed analysis of the oncogenic lesions that drive disease. One prominent oncogenic change seen in many cancers is somatic-activating mutations of tyrosine kinases, including BCR-ABL in chronic myelogenous leukemia (CML), mast/stem cell growth factor receptor (SCFR or KIT) in gastrointestinal stromal tumors (GIST), and EGFR in lung cancer (1–3). The dependency on tyrosine kinase activation in these tumors has led to successful clinical treatment with tyrosine kinase inhibitors (4–6). In prostate cancer, great progress has been made in identifying the genetic determinants of disease progression such as increased expression of androgen receptor (AR) and myelocytomatosis oncogene cellular homolog (MYC), phosphatase and tensin homologue deleted on chromosome 10 (PTEN) deletion, and erythroblast transformation specific (ETS) family gene fusions (7–11). However, recent large-scale cancer genome studies show that activating somatic mutations or DNA amplification of tyrosine kinase genes are rare in prostate cancer (8). This reveals why clinical administration of tyrosine kinase inhibitors for the treatment of advanced prostate cancer has been less effective and strongly implies that a more complete understanding of the tyrosine kinases that contribute to this disease is warranted (12, 13).

Despite the paucity of activating somatic mutations in tyrosine kinases, recent evidence suggests that tyrosine kinase phosphorylation in prostate cancer contributes to disease progression. In androgen-depleted conditions, tyrosine kinase, non-receptor, 2 (TNK2 or ACK1), SRC, and erythroblastic leukemia viral oncogene homolog 2 [ERBB2 (HER-2/neu)] tyrosine kinase activity can restore AR function in prostate cancer cells (14–17). Increased expression of the tyrosine kinase SRC and AR can synergistically drive frank carcinoma of the mouse prostate (18). This relationship results in robust activation of SRC tyrosine kinase and MAPK signaling (18). SRC activity was also observed in a subset of castration-resistant prostate cancer (CRPC) patients, which correlated with lower overall survival and increased metastatic disease (19). These data support the idea that tyrosine kinase activity may play a prominent role in prostate cancer progression in the absence of activating mutations.

Nearly 50% of tyrosine kinases are thought to contribute to human cancers, yet tyrosine phosphorylation represents less than 1% of the phosphoproteome (20). Sensitive and specific methods capable of enriching tyrosine phosphorylated peptides via antibody binding followed by quantitative mass spectrometry (MS) identification has become useful for the elucidation of tyrosine kinase signaling pathways, nodes, and negative feedback mechanisms in different cancer types (21–23). The ability to sensitively characterize pathway alterations in the presence of activated tyrosine kinases or tyrosine kinase inhibitors can allow for the identification of new potential drug targets (21, 24). We use this approach to identify and characterize tyrosine kinase signaling networks in transformed tissues that do not express mutated tyrosine kinases.

Global tyrosine phosphorylation in clinical prostate cancer samples was measured by immunohistochemistry (IHC) and showed a substantial increase in tyrosine phosphorylation in late-stage disease. To study this in a controlled manner, we evaluated tyrosine phosphorylation in a mouse model of prostate cancer progression using oncogenes common to prostate tumorigenesis and observed robust tyrosine phosphorylation in the advanced tumor phenotypes. Unbiased phosphotyrosine proteomics was used to investigate the specific tyrosine kinase signaling pathways activated by each of the nontyrosine kinase oncogenes. Analysis of the tyrosine phosphoproteome of these tumors revealed oncogene-specific tyrosine kinase activation including EGFR, ephrin type A receptor 2 (EPHA2), JAK2, ABL1, and SRC.

Results

Tyrosine Phosphorylation Is Increased in Clinical Castration-Resistant Prostate Cancer Samples. We performed IHC staining of prostate cancer tissue microarrays with the tyrosine phosphorylation-

MEDICAL SCIENCES

Fig. 1. Robust phosphotyrosine expression is observed in castration-resistant prostate cancer (CRPC) specimens. (*A*) Representative image of immunohistochemical staining using the phosphotyrosine-specific antibody, 4G10, of prostate specimens ranging from normal to CRPC. Tissue spots from patients with CRPC show high levels of phosphotyrosine expression in the epithelial compartment. (*B*) Increased tyrosine phosphorylation is observed in CRPC, with nearly 50% of the patients displaying high-intensity staining (2, 3) compared with normal, HGPIN, or HNPC tissues. (*C*) Average staining intensity of all of the tissues clearly show a significant increase of tyrosine phosphorylation in CRPC patients. HGPIN, high-grade prostatic intraepithelial neoplasia; HNPC, hormone naïve prostate cancer; HRPC, hormone refractory prostate cancer. ***$P < 0.001$, one-way ANOVA. (Scale bar, 200 μm.)

specific antibody 4G10 to evaluate phosphotyrosine expression during disease progression. CRPC (androgen independent) exhibited a robust increase in phosphotyrosine staining intensity compared with benign prostate, the precursor lesion high-grade prostatic intraepithelial neoplasia (HGPIN), or hormone naïve (androgen dependent) prostate cancer (HNPC) (Fig. 1*A*). Analysis of these tissue microarray samples indicated that 44% of CRPC specimens stain for phosphotyrosine at moderate to high levels (staining intensity 2–3), whereas only 11% of normal, 2% of HGPIN, and 2% of HNPC tissues stain at this intensity (Fig. 1*B*). Further, the average staining intensity of all of the CRPC tissue samples was significantly increased by over twofold compared with the other clinical phenotypes (Fig. 1*C*). These data reveal that tyrosine phosphorylation is present and elevated in CRPC and raise the notion that systemic treatment of patients with this disease may induce this response.

Tyrosine Phosphorylation Is Robust in Mouse Models of Advanced Prostate Cancer. The observation of increased tyrosine phosphorylation in late-stage prostate cancer specimens raises the question of whether tyrosine kinase activity is evident in prostate cancer models that do not express mutated or amplified tyrosine kinases. We recapitulated different stages of prostate cancer ranging from prostate intraepithelial neoplasia (PIN) to adenocarcinoma using the prostate in vivo regeneration model system (25, 26). We chose four of the most commonly perturbed oncogenes in prostate cancer, both in androgen-dependent and -independent states: activated AKT (myristoylated AKT, resembling *PTEN* deletion, ~40–70% of prostate cancers), AR amplification (~20–60% of prostate cancers), ERG rearrangements (~40–70% of prostate cancers), and activated K-RAS (K-RASG12V, resembling RAS/RAF pathway activation, observed in ~40–50% of prostate cancers) (7, 8, 11, 27–30).

We infected total mouse prostate cells with AKT alone or in combination with each respective oncogene using a lentiviral vector delivery system (Fig. 2*A*) and evaluated the histological phenotype of the resulting tumors after 12 wk. These tumors displayed histological characteristics of PIN (AKT), well differentiated and less aggressive cancer (AKT/ERG), or adenocarcinoma (AKT/AR and AKT/K-RASG12V) (Fig. 2*B*). IHC and Western blot analysis confirmed ectopic expression of each oncogene (Fig. S1 *A* and *B*). IHC staining and Western blot analyses displayed a gradient of phosphotyrosine expression in these tumors ranging from low to undetectable levels of tyrosine phosphorylation in the normal and indolent lesions (mouse prostate, AKT, or AKT/ERG) to very high levels in the more advanced tumors (AKT/AR and AKT/K-RASG12V) (Fig. 2*B* and Fig. S2 *A* and *B*).

Phosphoproteomic Profiling Identifies Oncogene-Dependent Tyrosine Phosphorylation of Kinases and Phosphatases. We enriched for tyrosine phosphorylated peptides and performed quantitative label-

free MS to identify phosphopeptides that contribute to this increased tyrosine phosphorylation (21, 31). We identified 139 phosphopeptides corresponding to 102 proteins (Dataset S1). Statistical analysis (ANOVA, 0.2 cutoff) revealed differential phosphorylation of 116 phosphopeptides corresponding to 87 proteins across all of the tumor phenotypes. Unsupervised hierarchical clustering analysis identified unique and overlapping patterns of tyrosine phosphorylated peptides for each tumor type, with an increased abundance of tyrosine phosphorylation events observed in the more advanced tumors (AKT/AR and AKT/K-RASG12V) (Fig. 3*A* and Fig. S3). These data demonstrate oncogene-specific signatures of phosphotyrosine activation across the spectrum of prostate cancer progression.

From the MS data, the activation sites of several tyrosine kinases and protein phosphatases were identified in the specific tumor
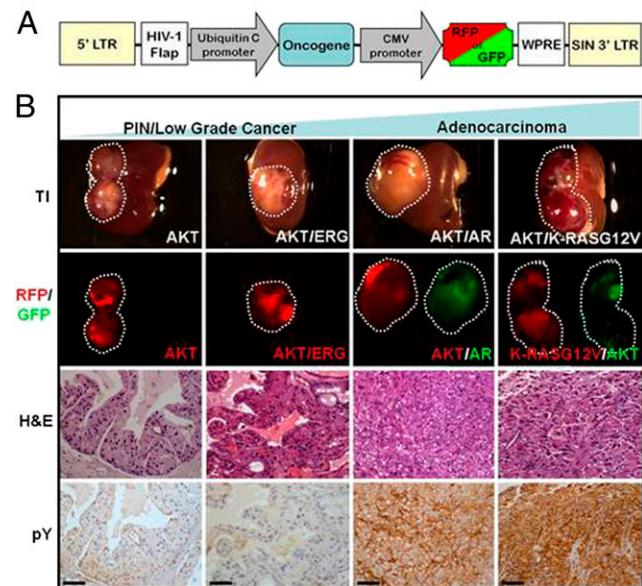

Fig. 2. Phosphotyrosine expression is increased during prostate cancer progression. (*A*) Lentiviral vector diagram displaying the organization of oncogene and fluorescent marker expression used in these tumors. (*B*) Gross and histological morphology of each tumor type after 12-wk engraftment in SCID mice using the prostate regeneration protocol. Fluorescence corresponds to expression of a particular oncogene. IHC staining of progressive mouse tumor phenotypes reveals an increasing gradient of phosphotyrosine expression with more aggressive tumors expressing higher levels than indolent tumors. TI, transillumination; H&E, hematoxylin and eosin; pY, phosphotyrosine. (Scale bars, 50 μm.)
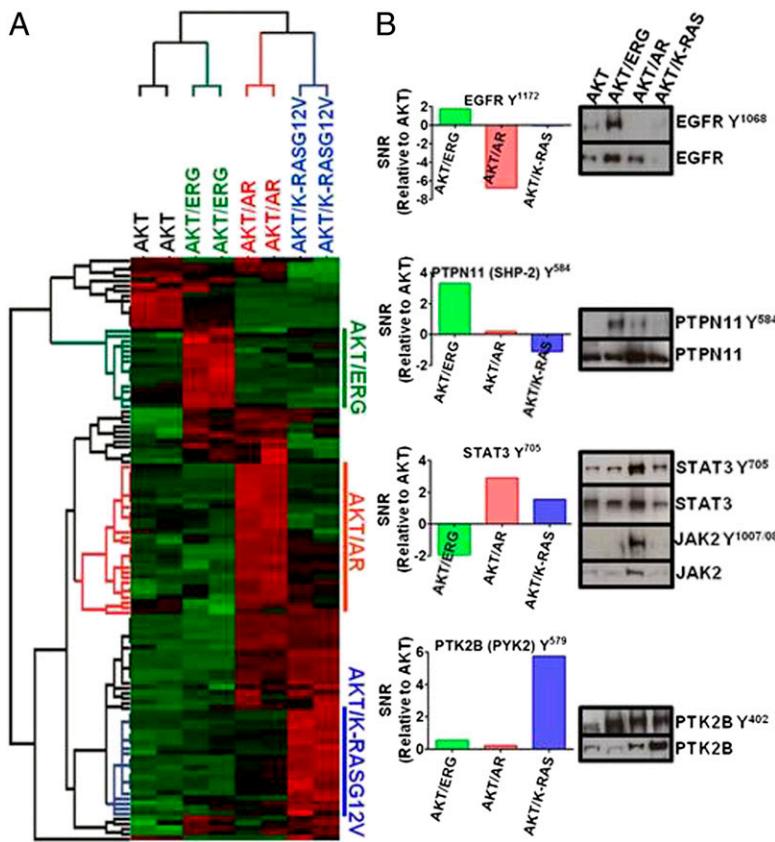
Fig. 3. Unique phosphotyrosine signatures are observed in a mouse model of prostate cancer progression. (*A*) Heatmap representing unique clusters of tyrosine phosphorylation for each mouse tumor phenotype. Each row corresponds to a unique phosphopeptide. Red, hyperphosphorylation; green, hypophosphorylation for each phosphopeptide. (*B*) Specific tyrosine kinases are observed in an oncogene-specific fashion. Signal-to-noise ratio (SNR) (relative to AKT) was calculated for each phosphorylation event and plotted. Positive SNR confirms elevation of that particular phosphorylation event. Western blotting validates indicated oncogene-specific phosphorylation results.

types (Table 1 and Figs. S3 and S4) (32). Consistent with these findings, Western blotting confirmed high levels of a second EGFR phosphorylation site (Y^{1068}) and PTPN11 (SHP-2) Y^{584} in AKT/ERG tumors (Fig. 3*B*). Activation of the JAK/STAT pathway was also revealed in AKT/AR tumors as high levels of phosphorylation of STAT3 Y^{705} were observed. Western blotting confirmed activation of the upstream kinase JAK2$^{Y1007/08}$ and STAT3 Y^{705} in this tumor type (Fig. 3*B*). We additionally identified an increase in phosphorylation of PTK2B/PYK2/FAK2 Y^{579} and Y^{849} in AKT/K-RASG12V tumors and Western blot confirmed the phosphorylation of the activation site Y^{402} of PTK2B (Fig. 3*B*). Together, these data demonstrate that each combina-

tion of prostate cancer oncogenes generates distinct patterns of tyrosine kinase and phosphatase activity.

Bioinformatic Inference of Tyrosine Kinase Activity Reveals Enrichment of Dasatinib Targets in AKT/AR Tumors. In addition to direct observation of phosphorylated tyrosine kinases and phosphatases by MS, we sought to use the phosphotyrosine peptide data to infer kinase activities specific to each tumor type. We predicted the activated kinases directly upstream for each observed phosphorylation site using known relationships from PhosphoSite (32), kinase motifs from PhosphoMotif Finder (33) and Phosida (34), and predictions from NetworKin (35). We then performed an

Table 1. Oncogene-specific phosphoactivation of tyrosine kinases and phosphatases

Oncogene combination	Tyrosine kinase (phosphoresidue)	Tyrosine phosphatase (phosphoresidue)	Functional significance*
AKT/ERG	EGFR (Y^{1172})	PTPN11 (Y^{584})	Enzymatic activation
		PTPRA (Y^{825})	Enzymatic activation
		INPPL1 (Y^{1136})	Unknown
AKT/AR	JAK2 ($Y^{1007/08}$)†	PTPN11 (Y^{62})	Enzymatic activation
AKT/KRASG12V	EPHA2 (Y^{595})		Enzymatic activation
	LYN (Y^{508})		Enzymatic inhibition
	PTK2B (Y^{579})		Unknown
	PTK2B (Y^{849})		Unknown
	EPHA2 (Y^{773})		Unknown
AKT/ERG	FER (Y^{402})		Alters cell motility
AKT/AR			
AKT/KRASG12V			

All measured phosphorylation events are relative to AKT-only lesions.
*Source: Phosphosite (http://www.phosphosite.org).
†JAK2 was not identified by MS, but inferred on the basis of high STAT3 Y^{705} phosphorylation observed in AKT/AR tumors.
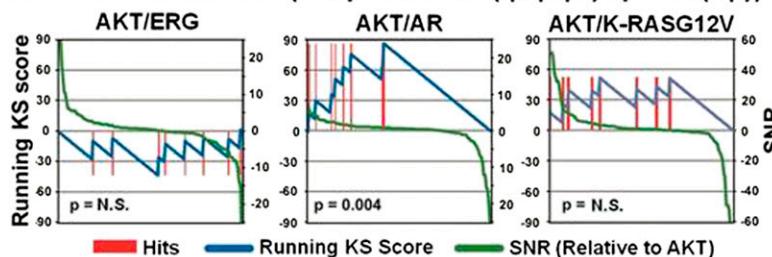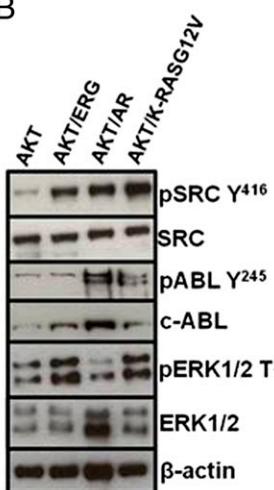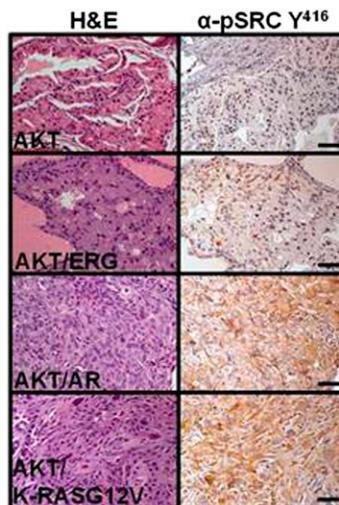
Fig. 4. Bioinformatic analysis reveals enrichment of dasatinib tyrosine kinase targets in AKT/AR tumors. (*A*) Enrichment analysis of tyrosine phosphosite motifs reveals enrichment of phospho-substrates of the tyrosine kinases ABL and SRC, targets of the tyrosine kinase inhibitor dasatinib, in AKT/AR tumors. No significant enrichment of these phosphopeptides were observed in either AKT/ERG or AKT/K-RASG12V tumors. Enrichment scores for all kinase motifs are shown in Dataset S2. (*B*) Western blot and (*C*) IHC staining for the activated kinases ABL, SRC, or ERK1/2 reveal tumor-specific activation of these kinases. (Scale bars, 50 μm.)

enrichment analysis of kinase-associated phosphorylation targets (*Materials and Methods*) to determine which kinase activities were predicted to be highly active in each tumor type.

Using this unbiased bioinformatic approach, we identified a statistically significant enrichment of the EGFR kinase substrate (D|E)pY in AKT/ERG but not in AKT/AR or AKT/K-RASG12V tumors (Fig. S5 and Dataset S2). Notably, this bioinformatic prediction was in direct agreement with our phospho-proteomic and Western blot data (Fig. 3*B*). Inference of kinase activity in AKT/K-RASG12V tumors further revealed an enrichment of ERK1/2 and MEK1/2 substrates, consistent with direct activation of MAPK signaling by the K-RASG12V oncogene (Fig. 4*B* and Fig. S4 and Dataset S2) (36).

Evaluating kinase activity from AKT/AR phosphopeptides revealed statistically significant enrichment of two motifs associated with ABL1 and SRC kinases [EXIpYXXP and (I|V|L|S) XpYXX(L|I), respectively] (37). Because these kinases are both targets of the tyrosine kinase inhibitor, dasatinib, we combined these motifs into a "dasatinib target" group and found enrichment of predicted ABL1 and SRC substrates in AKT/AR tumors (Fig. 4*A* and Dataset S2). AKT/K-RASG12V and AKT/ERG tumors demonstrated modest and no enrichment of these motifs, respectively. Western blotting and IHC validated this bioinformatic prediction, as both SRC Y^{416} and ABL1 Y^{245} were highly phosphorylated only in the AKT/AR tumor type, whereas SRC Y^{416} but not ABL1 Y^{245} were phosphorylated in AKT/ERG and AKT/K-RASG12V tumors (Fig. 4 *B* and *C*). This result demonstrates that substrate-based bioinformatic approaches for inferring kinase activity can reveal oncogene-specific tyrosine kinase activation not originally identified directly by phospho-MS.

Assembly of Oncogene-Specific Tyrosine Kinase Signaling Networks from Phosphoproteomic Data and Public Databases. We next sought to combine our phosphopeptide and bioinformatics data with information from public databases of protein–protein interactions (Human Protein Reference Database, HPRD) and posttransla-

tional modifications (Phosphosite) to manually construct tyrosine kinase signaling networks for each oncogene combination. In AKT/ERG tumors, identification of the EGFR substrate Y^{771} of phospholipase C, gamma 1 (PLCG1), and EGFR interacting proteins catenin, delta 1 (p120 catenin, CTNND1), PTPN11, and PTPRA, suggest strong association and activation of the EGFR tyrosine kinase pathway (Fig. 5). In AKT/AR tumors, detection of elevated SRC and ABL1 activity prompted us to investigate other substrates and binding partners of these kinases within our phosphoproteomic data. The identification of SRC and ABL1 substrates Y^{705} of STAT3, Y^{14} of caveolin-1 (CAV-1), and $Y^{1007/1008}$ of JAK2 with binding partners vinculin (VCL) Y^{822}, paxillin (PXN) Y^{118}, CTNND1 Y^{96}, and PTPN11 Y^{62}, suggest that, along with JAK2, these kinases act in concert toward the development of AKT/AR tumors (Fig. 5). The identification of the activation site of EPHA2 Y^{595} and downstream effectors ERK1 Y^{204} and ERK2 Y^{184} reveals strong MAPK activation in AKT/K-RASG12V tumors (Fig. 5). Further, the identification of VCL Y^{822} and PXN Y^{118} in AKT/AR and AKT/K-RASG12V tumors suggests that regulation of focal adhesions may be important for motility and survival in these tumors. The phosphorylation of PXN at Y^{118} by focal adhesion kinase (FAK) increases cell motility and survival, which are characteristic features of cells that have undergone an epithelial-to-mesenchymal transition (EMT) (38). The possibility of an EMT phenotype would be consistent with previous tumor phenotypes where SRC activation was observed (18). The manual curation of phosphotyrosine networks suggest novel associations of tyrosine kinase signaling with defined oncogenic insults in prostate cancer.

Discussion

Many studies have linked the aberrant activation of tyrosine kinases by somatic mutation or DNA amplification to a wide array of cancers (39, 40). We demonstrate oncogene-specific signatures of global phosphotyrosine activity without ectopic expression of mutant tyrosine kinases in a mouse model of prostate cancer progression. The activation of tyrosine kinase signaling suggests
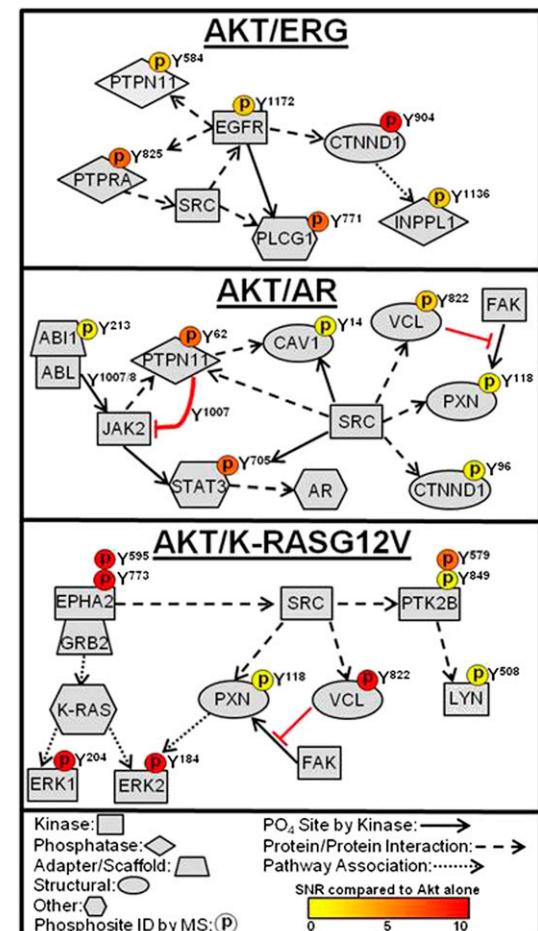
Fig. 5. Curation of phosphoproteomic profiling and bioinformatics delineates distinct tyrosine kinase signaling pathways in an oncogene-specific manner. Selected substrate and interaction pathways from each tyrosine kinase were generated from a combination of our phosphoproteomics dataset and the HPRD and Phosphosite databases. An elevated phosphorylation event identified by MS is indicated by a phosphorylation residue depicted above the protein and color coded. Solid arrow, protein is a direct substrate of the upstream kinase at that site. Dashed arrow, protein interacts directly with the upstream kinase/protein. Dotted arrow, protein is found within the pathway of the upstream kinase/protein.

the presence of alternative mechanisms regulating tyrosine kinase activity not related to activating mutations (18, 21, 22). These include but are not limited to loss of negative feedback mechanisms (e.g., increased or decreased phosphatase activity), transcriptional up-regulation of kinases, or increased stabilization of tyrosine kinases through decreased protein degradation (22, 41, 42). Our data suggest that some of these mechanisms may control tyrosine kinase signaling in our mouse model of prostate cancer.

Tyrosine phosphorylation of the protein tyrosine phosphatase PTPN11 may contribute to the phosphotyrosine signatures observed in our tumors. Activity of this phosphatase is often associated with increased signaling activity (43, 44). This phosphatase was highly phosphorylated on Y^{62} and Y^{584} in AKT/AR and AKT/ERG tumors, respectively. In EGFR-expressing fibroblasts, epidermal growth factor (EGF) stimulation resulted in Y^{584} phosphorylation of PTPN11 leading to RAS/ERK pathway activation (45). This supports our findings that Y^{584} of PTPN11 is highly phosphorylated in AKT/ERG tumors and suggests receptor tyrosine kinase pathway-mediated activation of PTPN11. PTPN11 inhibition leads to decreased xenograft growth of lung and prostate tumors and reduced activity of numerous tyrosine kinases, including SRC (46). PTPN11 $Y^{62/63}$ activation results in tyrosine

dephosphorylation of the inactive site of SRC Y^{530} by regulation of the Csk regulator PAG/Cbp, indicating that SRC activity in AKT/AR tumors may be dependent on PTPN11 activation (43, 46).

Transcriptional up-regulation of tyrosine kinases may also enhance tyrosine kinase activity as suggested by the phosphorylation of EPHA2 at Y^{595} in the AKT/K-RASG12V tumors. EPHA2 was shown to be a transcriptional target of the RAS–MAPK pathway and ligand-stimulated EPHA2 negatively regulates RAS activity (47). Constitutive activation of RAS through mutation bypasses the negative regulation of EPHA2 and results in increased MAPK activation, which is in direct agreement with our phosphoproteomic data. RAS activation may reveal why high expression levels of EPHA2 protein are observed in breast and prostate cancer and supports further clinical investigation of the connection between RAS mutation and EPHA2 status in these diseases (48, 49).

Tyrosine kinase activation offers therapeutic opportunities following the emerging successes of tyrosine kinase inhibitor therapies (5, 50). Our observation of SRC activity supports previous work that this kinase synergizes with other genes, including AR, to contribute to prostate adenocarcinoma (18, 51). SRC has also been shown to interact with the intracellular region of ERBB2 (HER-2), supporting the notion that SRC may be an important node for targeted therapy in advanced prostate cancer (17, 52). In support of these data, the SRC and ABL1 tyrosine kinase inhibitor dasatinib in combination with docetaxel is currently in phase III clinical trials for advanced prostate cancer and has shown modest phase I/II trial results in overall patient survival (53). Due to the heterogeneity of prostate cancer, this modest effect may be a result of the general administration of dasatinib without stratification of patients on the basis of SRC and ABL1 activity.

Strong activation of the EGFR pathway was observed in AKT/ERG-expressing mouse prostate tumors. Roughly half of all prostate cancer patients display the *TMPRSS2-ERG* translocation, a gene rearrangement fusing the androgen-regulated promoter of *TMPRSS2* with the ETS transcription factor *ERG*, which is considered to be a marker for prostate cancer progression from PIN to adenocarcinoma (54). The product of the *TMPRSS2-ERG* translocation was shown to interact with the enzyme poly (ADP ribose) polymerase 1 (PARP1), and inhibition of this enzyme abrogates growth of prostate cancer xenografts that ectopically express ERG (55). PARP1 inhibition represents a promising treatment option for patients with *TMPRSS2-ERG* translocations. Our data suggest that EGFR activity level is another candidate target in patients with *TMPRSS2-ERG* translocations. This result is in agreement with recent reports of SPINK1$^+$/ETS$^-$ prostate cancers where SPINK1-mediated growth occurs via EGFR signaling, demonstrating alternative pathways to activate EGFR (56). It will be important to further evaluate the relationship between EGFR activity and ERG clinically.

Our data suggest the molecular stratification of patients to target prostate cancer with tyrosine kinase inhibitors even in tumors without obvious tyrosine kinase mutations. Future work will extend this approach to prostate cancer patients to match tyrosine kinase inhibitor therapies with signaling activation patterns for targeted treatment of this disease.

Materials and Methods

Clinical Prostate Tissue Microarrays, *Lentiviral Vector Construction*, *Prostate Regeneration and Prostate Epithelial Viral Infections*, and *Western Blot and Immunohistochemistry* can be found in *SI Materials and Methods*.

Quantitative Analysis of Phosphotyrosine Peptides by Mass Spectrometry. A total of 300–500 mg of frozen tumor mass was homogenized and sonicated in urea lysis buffer (20 mM Hepes pH 8.0, 9 M urea, 2.5 mM sodium pyrophosphate, 1.0 mM β-glycerophosphate, 1% N-octyl glycoside, 2 mM sodium orthovanadate). A total of 35 mg of total protein was used for phosphotyrosine peptide immunoprecipitation as previously described (21, 57, 58). Additional details can be found in *SI Materials and Methods*.

Prediction of Kinase-Substrate Relationships. For each phosphopeptide, we predicted the potential upstream kinases using three types of data: (*i*) NetworKIN 2.0 kinase-substrate relationships (http://networkin.info/version_2_0/search.php), (*ii*) PhosphoSite kinase-substrate dataset (http://www.

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Non-Tyrosine Kinase Oncogenes Drive Distinct Tyrosine Phosphorylation Profiles in a Model of Progressive Prostate Cancer

Justin M. Drake, Nicholas A. Graham, Tanya Stoyanova, Amir Sedghi, Andrew S. Goldstein, Houjian Cai, Daniel A. Smith, Hong Zhang, Evangelia Komisopoulou, Jiaoti Huang, Thomas G. Graeber, Owen N. Witte

Abstract

Despite the numerous oncogenic alterations that are implicated in prostate cancer, dominant mutation or amplification of tyrosine kinases are not readily observed. While dominant acting kinases are absent in prostate cancer, recent literature implicates tyrosine kinase activity in advanced disease, although the mechanisms of how these kinases contribute to prostate cancer progression are largely unknown. Here, we observed increased tyrosine phosphorylation in a large subset of clinical prostate cancer patients with castration resistant disease or lymph node positive metastasis, raising the questions of whether tyrosine kinase activity is important for and which tyrosine kinases contribute to disease progression. Using the most common perturbed oncogenes and pathways in prostate cancer, we created different transformation phenotypes of this disease and investigated whether tyrosine kinase activity is observed in these tumors. Interestingly, we detected significant tyrosine phosphorylation in the tumor types with adenocarcinoma, without ectopic overexpression or mutation of any tyrosine kinase, when compared to indolent tumors displaying hyperplasia or prostate intraepithelial neoplasia (PIN). To identify the phosphorylated proteins, we enriched for tyrosine phosphorylated peptides followed by their identification using mass spectrometry. This approach, in addition to the prediction of substrate/kinase relationships, revealed a statistical enrichment of substrate targets of the tyrosine kinase inhibitor, dasatinib, in the more aggressive tumor types. Based on this enrichment, we evaluated the efficacy of dasatinib on tumors not expressing a dominant kinase using FDG-PET imaging. Taken together, identification of specific tyrosine phosphorylated peptides from genetically-defined tumor models suggests that treating patients via tyrosine kinase nodes even when tyrosine kinases are not amplified or mutated.

Therapeutic targeting of oncogene-specific tyrosine kinase networks during prostate cancer progression

Justin M. Drake, Nicholas A. Graham, Tanya Stoyanova, Amir Sedghi, Andrew S. Goldstein, Houjian Cai, Daniel A. Smith, Hong Zhang, Evangelia Komisopoulou, Jiaoti Huang, Thomas G. Graeber, Owen N. Witte

Abstract

Prostate cancer is the most highly diagnosed and second leading cause of cancer related death in American men. Despite the numerous oncogenic alterations implicated in prostate cancer, dominant activating mutations or DNA amplification of tyrosine kinases are not readily observed. We demonstrate that castration resistant prostate cancer (CRPC) exhibits increased tyrosine phosphorylation, raising the question of whether enhanced tyrosine kinase activity is observed in this disease in the absence of specific tyrosine kinase mutations. We generated a mouse model of prostate cancer progression using combinations of commonly perturbed non-tyrosine kinase oncogenes and pathways, including myristoylated Akt (Akt), androgen receptor (AR), Erg, and activated Kras (KrasG12V). We recapitulated the different stages of prostate cancer ranging from the precursor lesion prostatic intraepithelial neoplasia (PIN) (Akt) to low grade cancer (Akt+Erg) to highly aggressive prostatic adenocarcinoma (Akt+AR or Akt+KrasG12V). We detected a significant upregulation of tyrosine phosphorylation at the advanced adenocarcinoma stage. Phosphotyrosine peptide enrichment and quantitative mass spectrometry identified oncogene-specific tyrosine kinase signatures, including activation of Egfr Y^{1172} (Akt+Erg), EphA2 Y^{595} (Akt+KrasG12V), and Jak2 $Y^{1007/08}$ (Akt+AR). Kinase:substrate relationship analysis of the phosphopeptides also revealed Abl Y^{245} and Src Y^{416} tyrosine kinase activation in Akt+AR tumors; known targets of the tyrosine kinase inhibitor Dasatinib. We are currently evaluating the efficacy of tyrosine kinase inhibitors on non-tyrosine kinase driven tumors. In summary, the observation of elevated phosphotyrosine signaling in advanced prostate cancer and identification of specific tyrosine kinase pathways from genetically-defined tumor models points to new therapeutic approaches using tyrosine kinase inhibitors for advanced prostate cancer.

2012 AACR Advances in Prostate Cancer Research Program

**Justin M Drake presentation highlighted in <mark>yellow</mark>

* - Short talks from proffered papers

Opening Keynote Lectures

Session Co-Chairpersons: Charles L. Sawyers, Memorial Sloan-Kettering Cancer Center, New York, NY, and Arul M. Chinnaiyan, University of Michigan, Ann Arbor, MI
6:30 p.m.-8:00 p.m.
[6:30-7:15]
COP1: Tumor suppressor role revealed
Vishva M. Dixit, Genentech Inc., South San Francisco, CA
[7:15-8:00]
Polycomb dependent and independent functions of EZH2 in prostate cancer
Myles Brown, Dana-Farber Cancer Institute, Boston, MA

Networking Reception
8:00 p.m.-9:30 p.m.

Continental Breakfast
7:30 a.m.-8:30 a.m.

Session 1: Genomics / Molecular Profiling

Session Chairperson: Elaine Mardis, Washington University School of Medicine, St. Louis, MO
8:30 a.m.-10:00 a.m.
[8:30-9:00]
Genomic comparisons of coincident prostate cancer foci
Elaine Mardis
[9:00-9:30]
Functional implications of relocation of regulated transcription units in prostate cancer
M. Geoffrey Rosenfeld, University of California, San Diego, La Jolla, CA
[9:30-10:00]
Outcome prediction from the pattern of aberrations in prostate cancer genomes
Barry S. Taylor, Memorial Sloan-Kettering Cancer Center, New York, NY

Break
10:00 a.m.-10:20 a.m.

Session 2: Genomics / Molecular Profiling: Prognostic Signatures

Session Chairperson: Elaine Mardis, Washington University School of Medicine, St. Louis, MO
10:20 a.m.-11:20 a.m.
<u>PLEASE NOTE:</u> *This session is not accredited for CME credit.*
[10:20-10:40]
Biological pathways predictive of clinically significant prostate cancer in the context of tumor heterogeneity
Mark Lee, Genomic Health, Redwood City, CA
[10:40-11:00]
Cell cycle progression genes differentiate indolent from aggressive prostate cancer
Steve Stone, Myriad Genetics, Salt Lake City, UT
[11:00-11:20]
Development of integrated diagnostic methods for tissue testing
Gary Pestano, Ventana/Roche Group, Oro Valley, AZ

Session 3: Androgen Receptor Signaling

Session Chairperson: Karen Knudsen, Jefferson University Hospital, Philadelphia, PA
11:20 a.m.-12:50 p.m.
[11:20-11:50]
Mechanisms regulating distinct AR transcriptional programs in PCa
Steven P. Balk, Beth Israel Deaconess Medical Center, Boston, MA

[11:50-12:20]
Cross talk of the androgen receptor and DNA damage pathways: Molecular and translational prostate cancer relevance
Karen E. Knudsen
[12:20-12:50]
Overcoming castration-resistant prostate cancer
Charles L. Sawyers, Memorial Sloan-Kettering Cancer Center, New York, NY

Poster Session A / Lunch
12:50 p.m.-3:20 p.m.
Click here for a list of posters scheduled in this session

Session 4: Drug Development
Session Chairperson: Johann S. de Bono, The Institute of Cancer Research and Royal Marsden Hospital, Sutton, United Kingdom
3:20 p.m.-4:50 p.m.
[3:20-3:50]
Immune checkpoint blockade in prostate cancer: New insights and opportunities
James P. Allison, Memorial Sloan-Kettering Cancer Center, New York, NY
[3:50-4:20]
Cabozantinib (XL-184) and prostate cancer: Preclinical and clinical profile of a novel agent
Maha Hussain, University of Michigan Medical School, Ann Arbor, MI
[4:20-4:50]
Conducting hypotheses testing trials for the treatment of advanced prostate cancer
Johann S. de Bono

Poster Session B / Reception
5:00 p.m.-7:30 p.m.
Click here for a list of posters scheduled in this session

Dinner on own / Evening off
7:30 p.m.-

WEDNESDAY, FEBRUARY 8

Continental Breakfast
7:30 a.m.-8:30 a.m.

Session 5: ETS Gene Fusions
Session Chairperson: Mark A. Rubin, Cornell University Weill Medical College, New York, NY
8:30 a.m.-10:00 a.m.
[8:30-9:00]
Recurrent *SPOP* mutations define a distinct molecular subclass of prostate cancer
Mark A. Rubin
[9:00-9:30]
Novel therapeutic targets in prostate cancer
Arul M. Chinnaiyan, University of Michigan, Ann Arbor, MI
[9:30-9:45]
Context-specific oncogenesis by ETS-family transcription factors
Yu Chen, Memorial Sloan-Kettering Cancer Center, New York, NY
[9:45-10:00]
PARP1 inhibition as a strategy for targeting ETS gene fusions
Felix Feng, University of Michigan, Ann Arbor, MI

Break
10:00 a.m.-10:20 a.m.

Session 6: Prostate Cancer Initiation and Progression
Session Chairperson: Michael Shen, Columbia University Medical Center, New York, NY
10:20 a.m.-11:50 a.m.
[10:20-10:50]
Stem cells and the origin of prostate cancer
Michael Shen
[10:50-11:20]
Mechanisms and therapeutic targets in metastatic prostate cancer

Karen Cichowski, Brigham and Women's Hospital, Cambridge, MA
[11:20-11:50]
Stem cells and prostate cancer
Owen N. Witte, University of California, Los Angeles, CA

Poster Session C / Lunch
12:00 p.m.-2:30 p.m.
Click here for a list of posters scheduled in this session

Session 7: Late-Breaking Research
Session Co-Chairpersons: Charles L. Sawyers, Memorial Sloan-Kettering Cancer Center, New York, NY, and Arul M. Chinnaiyan, University of Michigan, Ann Arbor, MI
2:30 p.m.-5:00 p.m.
[2:30-2:45]
Altered AR gene architecture and splicing in castration-resistant prostate cancer
Scott M. Dehm, University of Minnesota, Masonic Cancer Center, Minneapolis, MN
[2:45-3:00]
Cooperation between androgen receptor and polycomb in prostate cancer
Jindan Yu, Northwestern University Lurie Comprehensive Cancer Center, Chicago, IL
[3:00-3:15]
A bad influence: ERG, AR, and prostate cell differentiation
Raymond A. Pagliarini, Novartis Institutes for BioMedical Research, Cambridge, MA
[3:15-3:30]
Transcriptional programs directed by the androgen receptor splicing variants
Jun Luo, Johns Hopkins University, Baltimore, MD
[3:30-3:45]
* Oncogenic ETS over-expression mimics RAS/MAPK signaling in prostate cells
Peter C. Hollenhorst, Indiana University, Bloomington, IN
[3:45-4:00]
* Optimization and applications of a tissue slice culture model of the normal and malignant human prostate
Sophia L. Maund, Stanford University, Stanford, CA
[4:00-4:15]
* The snoRNP assembly factor SHQ1 is a novel prostate cancer tumor suppressor gene
Phillip J. Iaquinta, Memorial Sloan-Kettering Cancer Center, New York, NY
[4:15-4:30]
* Biochemical recurrence is not a definitive surrogate endpoint for development of clinically useful predictive models for post-prostatectomy patients
Anamaria Crisan, GenomeDx, Vancouver, BC, Canada
[4:30-4:45]
* Therapeutic targeting of oncogene-specific tyrosine kinase networks during prostate cancer progression
Justin M. Drake, University of California, Los Angeles, CA
[4:45-5:00]
* Cabozantinib (XL184) inhibits androgen-sensitive and castration-resistant prostate cancer in the bone and increases bone formation in non-tumored bones
Eva Corey, University of Washington, Seattle, WA

Dinner on own / Evening off
5:00 p.m.-

THURSDAY, FEBRUARY 9

Continental Breakfast
7:30 a.m.-8:30 a.m.

Session 8: Imaging
Session Chairpersons: Martin G. Pomper, Johns Hopkins Kimmel Comprehensive Cancer Center, Baltimore, MD
8:30 a.m.-10:00 a.m.
[8:30-9:00]
Molecular imaging of androgen receptor signaling in CRPC
Steven M. Larson, Memorial Sloan-Kettering Cancer Center, New York, NY
[9:00-9:30]
Diagnosing prostate cancer with image fusion (MRI, PET, CT, US)
Peter Choyke, National Cancer Institute, Bethesda, MD
[9:30-10:00]
Molecular imaging agents for prostate cancer: Focus on PSMA
Martin G. Pomper

Break

10:00 a.m.-10:30 a.m.

Session 9: Closing Keynote Lectures

Session Co-Chairpersons: Arul M. Chinnaiyan, University of Michigan, Ann Arbor, MI, and Charles L. Sawyers, Memorial Sloan-Kettering Cancer Center, New York, NY
10:30 a.m.-11:30 a.m.
[10:30-11:00]
Inflammation as the trigger of somatic epigenome defects in prostate cancer
William G. Nelson, Johns Hopkins Kimmel Comprehensive Cancer Center, Baltimore, MD
[11:00-11:30]
Role of the microenvironment in prostate cancer progression and resistance
Christopher J. Logothetis, University of Texas MD Anderson Cancer Center, Houston, TX

Departure